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Evidence Supporting the use of Probiotics for the Prevention and Treatment of Chemotherapy-Induced Intestinal Mucositis

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Although chemotherapy remains the current best practice for the treatment of neoplasia, the severity of its associated side-effects continues to impact detrimentally on the quality of life. Mucositis can affect both the oral cavity and intestine, and represents one of the most common side-effects of chemotherapy. It is characterized by ulceration, inflammation, diarrhoea, and intense abdominal pain. Despite extensive research there remains no definitive therapy for mucositis. This may be due to the multiple factors which contribute to its pathogenesis, including up-regulation of pro-inflammatory cytokines, increased apoptosis of epithelial cells, alteration of the gastrointestinal microbiota, and damage to the epithelium. Although employed increasingly in other gastrointestinal disorders, probiotics are yet to be comprehensively investigated in the treatment or prevention of chemotherapy-induced mucositis. Probiotic-based therapies have been shown to exert beneficial effects, including modulation of the microbiota and inhibition of pro-inflammatory cytokines. This review outlines the current evidence supporting the use of probiotics in intestinal mucositis, and suggests further research directions for the future.

Keywords cancer, functional foods, gastrointestinal tract

INTESTINAL MUCOSITIS

Chemotherapy and radiotherapy are common, effective treatments for various forms of cancer. However, the cytotoxic effect of these treatments presents a major oncological problem (Gibson and Keefe, 2006; Tooley et al., 2006a; Logan et al., 2007). Radiotherapy and chemotherapy target rapidly dividing neoplastic cells, but can also affect the progenitor cell populations located in various sites throughout the human body (Duncan and Grant, 2003). The epithelia of the gastrointestinal tract are particularly susceptible hence radio- and chemotherapy can often lead to the development of mucositis (Grant and Duncan, 2003; Gibson and Keefe, 2006; Tooley et al., 2006a; 2006b; Triantafyllou et al., 2008). Mucositis is a common disorder, with approximately 40% of patients receiving standard-dose chemotherapy, and almost 100% of patients receiving high-dose chemotherapy, being diagnosed with the condition (Keefe et al., 1997). Symptoms of gastrointestinal mucositis include severe inflammation, ulceration, lesioning, abdominal bloating, diarrhoea, nausea, and intense abdominal pain (Sonis et al., 2004; Logan et al., 2007).

The pathogenesis of intestinal mucositis remains undefined. It was originally hypothesized that intestinal damage occurred solely as a result of increased intestinal epithelial cell apoptosis due to chemotherapy treatment; however, recent theories suggest an important role for pro-inflammatory cytokines in the development of the disorder (Sonis, 1998). This proposed mechanism comprises five overlapping stages (Sonis, 1998; Scully et al., 2003). The first stage begins immediately following treatment with cytotoxic agents, involving indirect tissue

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damage as a result of the production of reactive oxygen species. The second stage is associated with the activation of transcription factors, most importantly nuclear factor-κB (NF-κB) (Logan et al., 2007). NF-κB activation promotes the up-regulation of genes which disrupt mucosal integrity, including the pro-inflammatory cytokines, tumor necrosis factor (TNF), and interleukin (IL)-1β and IL-6 (Sonis, 2002). In the third stage, pro-inflammatory cytokines act via positive feedback to induce further activation of NF-κB and, hence, further pro-inflammatory cytokine production. Recent investigations have shown that other biologically active proteins or pro-inflammatory mediators are also up-regulated during this stage, resulting in an inflammatory cascade and the activation of matrix metalloproteinases, leading to further epithelial damage (Sonis, 2002). It is only at the fourth stage of mucositis that the condition becomes clinically evident, as damage to the epithelial wall facilitates bacterial colonization, resulting in significant pain to the patient. This stage also involves the loosening of tight junctions in the epithelial wall, and the subsequent loss of barrier function, facilitating the transfer of harmful luminal antigens into the surrounding intestinal tissue (Keefe et al., 2000). The final phase occurs following discontinuation of cancer therapy, and is associated with re-epithelialization of the mucosa and the gradual return to typical mucosal appearance and function.

Recently, there has been a proliferation of studies which suggest that the gastrointestinal microbiota and mucins are altered by chemotherapeutic agents and may be involved in the development of mucositis (Stringer et al., 2007a; 2009a; 2009b). Changes to the composition of the microbiota can have serious implications for the host as it is involved in a number of important functions including maintenance of immunity, protection from pathogenic invasion, and nutrient processing, all of which may be compromised by chemotherapy. Chemotherapy-induced alterations to the gut microbiota are yet to be investigated in great detail; however, recent literature suggests that effects occur in an agent- and organ-specific manner (Stringer et al., 2009a). For example, 5-Fluorouracil (5-FU) administration has been reported to decrease *Clostridium spp.*, *Staphylococcus spp.,* and *Escherichia spp.* in the stomach, whereas in the jejunum, a decrease in *Lactobacillus spp.* and *Clostridium spp.* coincided with an increase in *Escherichia spp.* Colonic *Lactobacillus spp.* decreased, but both *Escherichia spp.* and *Clostridium spp.* were increased in the large intestine (Stringer et al., 2009b). In contrast, Irinotecan decreased *Enterococcus spp.*, *Serratia spp.*, and *Peptostreptococcus spp.* in the stomach, increased *Enteroccoccus spp.*, *Serratia spp.* (perhaps due to bacterial overflow from the stomach), *Lactobacillus spp.*, and *Clostridium spp.* in the jejunum; and increased colonic *Escherichia spp.* and *Clostridium spp.* (Stringer et al., 2007b).

Treatment with 5-FU has been shown to decrease goblet cell numbers, and increase mucin-secreting cavitated cell numbers in the crypts of the jejunum (Stringer et al., 2009b). These changes could inhibit the protective capabilities of the mucosal barrier following the depletion of stored mucins (Stringer et al., 2009b), thereby rendering the gut more susceptible to chemotherapy damage and subsequent pathogenic invasion.

PROBIOTICS

With the identification of the microbial environment and cytokine expression as key components of intestinal mucositis, probiotics represent a promising therapeutic option. Probiotics can be defined as live bacteria which, when administered in sufficient numbers, are able to exert beneficial physiologic or therapeutic activities (Sartor, 2004). Bacteria can be derived from various sources such as cultured food and the normal human microbiota, but must meet certain criteria including complete identification at genus, species, and strain level; production of antimicrobial substances; adherence to mucosal surfaces; safety for consumption; and stability during processing and storage (Borchers et al., 2009). Probiotic bacteria are most commonly of the *Lactobacillus* or *Bifidobacterium* genera although strains have also been identified from *Enterococcus*, *Streptococcus*, and *Lactococcus* species, while certain non-pathogenic *Escherichia* strains are also classified as probiotics (Borchers et al., 2009). Furthermore, probiotic strains can be genetically engineered to secrete specific bioactive compounds such as IL-10 (Steidler et al., 2000; Pang et al., 2009).

A high degree of species and strain specificity is associated with the beneficial effects exerted by probiotics, and as such the mechanisms underlying these effects are not completely understood. In addition to strain or species, mechanisms are also dependent on factors such as the bacterial environment and the disease setting under investigation (Shanahan, 2004). Common mechanisms of action identified in probiotics include inhibition of pathogenic enteric bacteria, improvement of epithelial barrier function, and manipulation of host immunoregulation (Sartor, 2004).

Rationale for Use of Probiotic-Based Therapies

Intestinal mucositis is characterized by a spectrum of deleterious effects on the gastrointestinal tract, including but not limited to uncontrolled inflammation (Sonis, 2002), increased intestinal permeability (Keefe et al., 2000), pathogen load (Stringer et al., 2009a), pro-inflammatory cytokine expression (Sonis et al., 2004; Logan et al., 2007), reduction of mucin levels (Stringer et al., 2009b), oxidative damage (Sonis, 1998), and increased cell apoptosis (Keefe et al., 2000). Interestingly, there is evidence, based largely on data from other intestinal disorders, to suggest that probiotics may be an effective method of treating each individual effect (Table 1) and thus, possibly mucositis as an entity.

Anti-Inflammatory Effects

Inflammation plays an important role in the development of intestinal mucositis. Treatment with anti-neoplastic agents activate the transcription factor NF-kB which in turn activates a number of pro-inflammatory cytokines (Logan et al., 2007). Certain probiotic strains have anti-inflammatory properties, and present a viable option for counteracting this component of

Table 1 Probiotic species, strains, or combination which have previously been shown to exert effects which suggest potential efficacy in either the treatment or prevention of chemotherapy-induced intestinal mucositis

Probiotic effect relevant to intestinal mucositis	Species/Strain/ Combination	Reference
Inhibition of inflammation	*F. prausnitzii*	Sokol et al., 2008
	L. plantarum	Petrof et al., 2004
	B. bifidum Yakult	Imaoka et al., 2008
	B. breve Yakult	Imaoka et al., 2008
	S. boulardi	Martins et al., 2009
Maintenance of intestinal permeability	VSL#3	Mennigen et al., 2009
	B. infantis	Ewaschuk et al., 2008
	L. rhamnosus GG	Seth et al., 2008
		Forsyth et al., 2009
Elimination of pathogenic bacteria	*L. plantarum* LP31	Muller et al., 2009
	L. plantarum 423	Ramiah et al., 2008
	L. rhamnosus GG	Collado et al., 2007
	L. johnsonii NCC533	Pridmore et al., 2008
Inhibition of cell apoptosis	VSL#3	Mennigen et al., 2009
	Ecologic®641	Lutgendorff et al., 2009
	S. boulardii	Dalmasso et al., 2006
	L. rhamnosus GG	Yan et al., 2007
Prevention of oxidative damage	Ecologic ®641	Lutgendorff et al., 2009
	L. fermentum CECT5716	Peran et al., 2007
	VSL#3	Esposito et al., 2009
Maintenance of mucus barrier	VSL#3	Caballero-Franco et al., 2007
	L. acidophilus	Kim et al., 2008
	L. plantarum 299v	Mack et al. 1999

intestinal mucositis. Sokol and colleagues (2008) recently isolated *Faecalbacterium prausnitzii*, a strain found in the microbiota of Crohn's disease patients associated with reduced risk of post-operative recurrence. This strain was investigated for anti-inflammatory properties both in vivo and in vitro. Stimulation of peripheral blood mononuclear cells with *F. prausnitzii* significantly reduced IL-12 and IFN-γ levels, and increased release of the anti-inflammatory cytokine, IL-10. Furthermore, in Caco-2 cells with a reporter gene for NF-kB activity, treatment with live bacteria had no effect; however, treatment with the bacterial supernatant completely inhibited NF-kB expression (Sokol et al., 2008). With the paramount role of NF-kB in the development of intestinal mucositis, probiotics capable of reducing NF-kB expression could be promising therapeutic candidates. Petrof and colleagues (2004) treated a mouse colon cell line with *Lactobacillus plantarum* conditioned media and reported reduced NF-kB binding activity and monocyte chemotactic protein-1 (MCP-1, an inflammatory chemokine involved in leukocyte recruitment, to areas of inflammation (MacDermott, 1999)) following activation by a TNF-receptor (Petrof et al., 2004). Conditioned media from *Bifidobacterium breve* Yakult and *Bifidobacterium bifidum* Yakult, administered individually, reduced pro-inflammatory IL-8 secretion in human HT-29 epithelial cells stimulated with TNF-α (Imaoka et al., 2008). Interestingly, only the *B. bifidum* Yakult conditioned media inhibited IL-8 gene expression in the cells, and as such the mechanism of action for *B. breve* Yakult remains obscure, but could be associated with the production of anti-inflammatory factors by probiotic bacteria. The ability to suppress expression of IL-8 (or other pro-inflammatory cytokines) presents a key mechanism by which probiotics may reduce the severity of intestinal mucositis.

In the trinitrobenzene sulfonic acid (TNBS) model of colitis, *F. prausnitzii* also demonstrated anti-inflammatory effects (Sokol et al., 2008), suggesting potential as a mucositis treatment. A six-day pre-treatment with either the live strain or its secreted compounds led to a reduction in colonic pro-inflammatory TNF-α and IL-12 levels, and an increase in anti-inflammatory IL-10. *Saccharomyces boulardii* has also been shown to increase in vivo levels of IL-10 in germ-free mice (Martins et al., 2009). Increasing levels of anti-inflammatory cytokines may be effective in the treatment of mucositis. However, a strain such as *F. prausnitzii* which can influence both pro- and anti-inflammatory pathways, may have more pronounced effects. Future studies comparing probiotics acting through different anti-inflammatory mechanisms for their capacity to treat or prevent intestinal mucositis should be investigated.

Roselli and colleagues (2009) tested two probiotic formulations—mix 1 consisted of *Lactobacillus acidophilus* Bar13 and *Bifidobacterium longum* Bar33 while mix 2 comprised *L. plantarum* Bar10, *Streptococcus thermophilus* Bar20, and *Bifidobacterium animalis* subsp. *lactis* Bar 30. Once again, the TNBS model of colitis was employed, and both combinations significantly increased levels of T regulatory cells and inhibited production of TNF-α and MCP-1. Furthermore, mix 1 inhibited IL-12 and IFN-γ release. However, individual strains were not investigated to determine if one particular strain was primarily responsible for the observed effects.

Maintenance of Intestinal Permeability

Damage to the epithelial wall is the first clinical sign of mucositis and can be particularly harmful to the patient (Sonis et al., 2004). Increased epithelial permeability allows the transfer of harmful pathogens into the surrounding tissue, and an overall loss of intestinal function (Keefe et al., 2000). A number of probiotic strains have been shown to improve integrity of the epithelium, allowing the intestinal barrier to maintain normal function.

Treatment with the live probiotic *Escherichia coli* Nissle 1917 resulted in an up-regulation of the tight junction molecule zonula occluden-1 at both mRNA and protein levels, and reduced intestinal barrier permeability in mice following dextran sulfate sodium (DSS)-induced damage (Ukena et al., 2007). The probiotic combination VSL#3 has also been successfully employed to prevent DSS-induced increases in intestinal permeability and decreases in expression of the tight junction proteins occludin and claudin-1, -3, -4, -5 in BALB/c mice (Mennigen et al., 2009). While the active bacterial strain(s) in VSL#3 was not determined in this study, the results of Ewaschuk and colleagues (2008) suggest that the *Bifidobacterium infantis* strain may have played an important role. Culture media (CM) from *B.*

infantis increased trans-epithelial resistance (TER, an indicator of intestinal integrity), ZO-1 and occludin expression in normal T84 cells. Furthermore, *B. infantis* CM prevented TNF-α and IFN-γ induced reduction of TER and rearrangements of tight-junction proteins. These findings were confirmed in vivo, as probiotic treatment normalized colonic permeability in IL-10-deficient mice (Ewaschuk et al., 2008). Although the ability of each individual strain to improve TER was determined, the authors did not compare *B. infantis* CM with either live VSL#3 or its CM. A future study comparing these effects would determine whether the ability of VSL#3 to improve intestinal permeability was due to a combination of strains, or *B. infantis* alone. The effects of four separate probiotic strains (*Bifidobacterium lactis* 420, *Bifidobacterium lactis* HN109, *Lactobacillus acidophilus* NCFM, and *Lactobacillus salivarius* Ls-33) on tight junction integrity (Putaala et al., 2008) have also been investigated. Differentiated Caco-2 cells were treated with cell-free supernatants of each strain. *B. lactis* 420 CM significantly increased TER suggesting that live bacteria are not always required to exert beneficial effects. These results are supported by earlier findings which indicated that soluble proteins produced by *Lactobacillus rhamnosus* GG were able to protect against hydrogen peroxide-induced epithelial damage (Seth et al., 2008). A separate study using *L. rhamnosus* GG in alcohol-induced gut leakiness provided further evidence of the ability of the strain to improve gut permeability (Forsyth et al., 2009). In this study, rats fed alcohol and treated with live probiotic had significantly reduced gut leakiness compared to alcohol-fed controls. Future studies should compare the effect of live *L. rhamnosus* GG to its cell-free supernatant in order to determine whether the effects are mediated entirely by secreted factors. The effectiveness of LGG in multiple forms of gut damage suggests potential for its use in the treatment of chemotherapy-induced intestinal damage.

Elimination of Pathogenic Bacteria

The role of pathogenic bacteria in intestinal mucositis is not yet completely defined; however, recent findings suggest that pathogenic bacteria play a key role in the development of the disorder (Stringer et al., 2007a; 2009a; 2009b). Chemotherapy has been shown to have a direct toxic effect on commensal bacteria (Stringer et al., 2009b). These changes contribute to the improved survival of pathogenic bacteria which, when combined with increased intestinal permeability and impaired immunity, render mucositis sufferers increasingly susceptible to infection (Stringer et al., 2009b). Probiotics have demonstrated the capacity to inhibit the survival of pathogens in the microbiota, suggesting a further mechanism by which they could prevent mucositis.

By binding to both epithelial cells and the mucus layer throughout the gastrointestinal tract, probiotics can prevent pathogen colonization by competitive exclusion (Candela et al., 2008). In a condition such as mucositis, where large-scale changes to the bacterial population occur (Stringer et al., 2007b; 2009b), probiotics, which are able to competitively-inhibit multiple pathogenic strains, are optimal. An example of such a strain is *L. plantarum* 423, which inhibited adhesion of pathogenic *Clostridium sporogenes* and *Enterococcus faecalis* (Ramiah et al., 2008). Moreover, *L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* 99, and *Propionibacterium freudenreichii* ssp. *Shermanii* JS represent further examples of probiotic strains capable of inhibiting multiple pathogens (Collado et al., 2007).

Specific probiotic strains also produce anti-microbial substances which target and eliminate pathogens from the gastrointestinal tract. *Lactobacillus johnsonii* NCC533 La1 was recently demonstrated to secrete anti-bacterial hydrogen peroxide(Pridmore et al., 2008). Both the live bacteria and its secreted compounds were able to eliminate the pathogenic *Salmonella enterica serovar typhimurium* SL1344. Hydrogen peroxide production only occurred in an aerobic environment and, as such, the anti-microbial activity of this strain may have been limited to the anaerobic human microbiota (Pridmore et al., 2008). Bacterial strains with broad-spectrum anti-bacterial effects are commonly reported and warrant further investigation in the setting of intestinal mucositis. Muller and colleagues (2009) demonstrated that *L. plantarum* LP31 exhibited a bactericidal effect against *Pseudomonas* sp., *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*. In an investigation of four separate *L. reuteri* strains, Spinler and colleagues (2008) determined that each strain produced the anti-microbial compound reuterin, and was effective at inhibiting the growth of enteric pathogens which included enterohemorrhagic *Escherichia coli*, enterotoxigenic *E. coli*, *Salmonella enterica*, *Shigella sonnei*, and *Vibrio cholerae*.

Prevention of Cell Apoptosis

An increase in the apoptosis/proliferation ratio is a common feature of intestinal mucositis and plays a key role in the development of the disorder as it leads to increased permeability of the epithelial wall (Sukhotnik et al., 2008). Probiotic administration has been shown to both inhibit and promote apoptosis in a variety of settings. Treatment with live VSL#3 significantly reduced caspase-3 (a positive marker of apoptosis (Bowen et al., 2006)) activation in the colon of rats with DSS-induced colitis (Mennigen et al., 2009). The ability of the probiotic combination to reduce apoptosis in this chemically-induced model of damage suggests potential efficacy in similar settings, such as chemotherapy-induced mucositis. With the exception of irinotecan-induced apoptosis (Bowen et al., 2007), the initiation of caspases in healthy intestinal tissues by chemotherapy drugs remains largely undefined. Future studies should further characterize the role of caspases in chemotherapy-induced apoptosis, allowing for the identification of probiotic strains with the ability to reduce caspase activation. In addition, the probiotic cocktail, Ecologic® 641, comprising four *Lactobacillus* and two *Bifidobacterium* strains, reduced cellular apoptosis in rats suffering from glycodeoxycholate-induced acute

pancreatitis (Lutgendorff et al., 2009). Neither study compared the antiapoptotic effects of their probiotic combinations in different areas of the gastrointestinal tract and thus, the degree of site specificity remains unknown. This characteristic may play a key role in the selection of a candidate probiotic strain for the treatment of mucositis. Probiotic strains or combinations which only reduce the apoptotic ratio in areas damaged by chemotherapy could be therapeutically effective.

Individual probiotic strains have also demonstrated anti-apoptotic effects. Pre-treatment with *S. boulardii* significantly inhibited TNF-α-induced apoptosis in human colonic T84 cells infected with pathogenic *Escherichia coli* (EHEC) (Dalmasso et al., 2006). These authors investigated the pathways via which EHEC initiated apoptosis and discovered that two different pathways were involved. The first involved death receptors and was identified by the activation of caspase-8, while the second comprised a number of intra- and extracellular death stimuli which led to the activation of caspase-9. These two pathways converge to trigger caspase-3. Treatment with *S. boulardii* blocked apoptosis by both pathways, leading to an overall inhibition of caspase-3 activation (Dalmasso et al., 2006). Reports of therapeutic agents inhibiting these pathways in mucositis are limited; however, a trial examining glucagon-like peptide-2 (GLP-2) demonstrated the importance of caspase-8 activation in irinotecan-induced apoptosis (Boushey et al., 2001). Inhibition of caspase-8 was shown to be a key mechanism via which GLP-2 enhanced the survival of epithelial cells following chemotherapy. The ability of *S. boulardii* to exert similar protective effects should be investigated. *L. rhamnosus* GG has also been shown to exert anti-apoptotic effects in a model of cytokine-induced apoptosis (Yan et al., 2007). Interestingly, Yan and colleagues compared the probiotic in its live form with two proteins (p40 and p75) isolated from the probiotic supernatant. Co-culture with TNF induced apoptosis in KSRI^{-}/$^{-}$MCE mouse colon cells (detected via TUNEL staining), but this was inhibited following treatment with live *L. rhamnosus* GG. Furthermore, apoptosis was also inhibited by co-culture with the two LGG-derived proteins, suggesting that these factors present in the supernatant are involved in the anti-apoptotic process. TNF-stimulated caspase-3 activity was also found to be reduced following co-culture of colonic tissue explants with p40 and p75; however, the initiator pathway involved in the activation was not determined. These findings suggest that the secreted factors of probiotics have the potential to be efficacious in the treatment of intestinal mucositis, and should be explored further.

While anti-apoptotic effects are common amongst probiotics, pro-apoptotic effects have also been reported (Myllyluoma et al., 2008). *L. rhamnosus* GG, *L. rhamnosus* Lc705, *B. breve* Bb99, and *P. freudenreichii* subsp. *shermanii* JS were all tested, individually and in combination, for their ability to inhibit *Helicobacter pylori*-induced apoptosis in differentiated Caco-2 cells. At 24 h, *H. pylori* infection significantly increased caspase-3 activation. Co-culture of cells with *H. pylori* and the probiotic combination reduced caspase-3 activity, as did treatment with *L. rhamnosus* LGG and *L. rhamnosus* Lc705 individually. No effect was observed following co-culture of *P. freudenreichii* subsp. *shermanii* JS and *H. pylori*, while culture of Caco-2 cells with *P. freudenreichii* subsp. *shermanii* JS alone led to a significant increase in caspase-3 activity. Although a rare occurrence, probiotics should be screened for any pro-apoptotic effects prior to investigation as a potential therapy for intestinal mucositis, to eliminate any risk of exacerbating the condition. Furthermore, the probiotics must not protect neoplastic cells from chemotherapy-induced apoptosis, as this would impede the effectiveness of the primary therapy.

Maintenance of Mucus Barrier

Mucins play a number of vital roles in the gastrointestinal tract, including the protection of the mucosa from bacterial overgrowth, providing attachment sites for intestinal flora and protecting the epithelium from luminal factors (Stringer et al., 2009b). Chemotherapy regimens have been shown to alter mucin dynamics, potentially reducing intestinal barrier function (Stringer et al., 2009b) and contributing to the onset of diarrhoea (Gibson et al., 2003). Ideally, candidate probiotics should be able to reduce the severity of chemotherapy-induced damage by maintaining the production of mucins by intestinal epithelial cells.

VSL#3 was tested both in vivo and in vitro for its ability to induce mucin secretion (Caballero-Franco et al., 2007). In vivo, mucin content, mucin secretion, and gene expression were all increased following VSL#3 administration. In contrast, treatment of human colonic LS 174T cells with live VSL#3 had no effect in vitro. Interestingly, treating cells with products secreted by VSL#3 also significantly increased mucin expression (Caballero-Franco et al., 2007). Furthermore, when the individual live strains of VSL#3 were compared to one another and the combination, secreted products from *Lactobacillus* species were the most potent mucin stimulators; however, the combination was still the greatest potentiator of mucin secretion. Kim and colleagues (2008) demonstrated that *L. acidophilus* A4 was able to increase expression of the mucin polypeptide MUC2 in vitro, and in turn inhibit binding of *E. coli* 0157:H7. However, the authors also reported an increase in IL-8, IL-1β, and TNF-α as a result of probiotic treatment, and it therefore remains unclear whether the reduced binding was due to mucin production, cytokine expression, or both. Similarly, increased MUC2 and MUC3 expression and an inhibition of pathogenic *E. coli* 0157:H7 binding to HT-29 cells was reported following co-incubation with *L. plantarum* 299v (Mack et al., 1999). As co-incubation of the pathogenic strain with the probiotic did not alter viability of *E. coli*, authors dismissed any anti-bacterial activity of the probiotic. However, earlier findings of Kim et al. (2008) suggest that cytokine and/or other immune responses to probiotic treatment should also be investigated. Future studies should also determine the correlation between mucin gene expression and mucin secretion to ensure that mRNA levels are an accurate representation.

Prevention of Oxidative Damage

The release of reactive oxygen species is hypothesized to play a role in the initial stages of mucositis, leading to the oxidative damage of intestinal tissue (Sonis, 1998; Scully et al., 2003). Inhibiting the release of reactive oxygen species could therefore reduce the overall severity of mucositis. Probiotics have demonstrated anti-oxidant effects in a number of models of oxidative damage and could be effective in reducing both initial tissue damage, and the subsequent host inflammatory response. A rat model of acute pancreatitis was used to demonstrate the anti-oxidant capabilities of the multispecies probiotic combination, Ecologic®641 (Lutgendorff et al., 2009). Five day pre-treatment with probiotics prevented the acute pancreatitis-induced reduction in lipid peroxidation and mucosal glutathione levels. Interestingly, probiotic therapy also increased mucosal glutathione levels compared to normal controls by up-regulating glutamate-cysteine-ligase activity (a rate-limiting component of glutathione biosynthesis). The effectiveness of a pre-treatment in preventing oxidative damage is particularly relevant in intestinal mucositis, a disorder which is induced deliberately and the time course of disease progression easily predicted. Peran and colleagues (2007) tested probiotic effects on glutathione levels, employing *Lactobacillus fermentum* CECT5716 in a model of TNBS-induced colitis. Probiotic pre-treatment prevented colonic glutathione depletion, although the mechanism behind this protection was not determined. Furthermore, *L. fermentum* reduced nitric oxide synthase expression, a further anti-oxidative effect.

The anti-oxidant capabilities of VSL#3 have also been reported in rats fed a high fat diet (Esposito et al., 2009). Probiotic treatment reduced expression of inducible nitric oxide synthase, protein nitrosylation, and malondialdehyde levels, all indicators of oxidative damage. This study, along with that of Lutgendorff et al. (2009), suggests that probiotic combinations may have applications in mucositis treatment. Future studies should attempt to determine which strains are primarily responsible for any observed effects, as this may facilitate the development of more targeted therapies.

RISKS ASSOCIATED WITH THE USE OF PROBIOTIC BASED THERAPIES

Although probiotics are considered as harmless bacteria which convey beneficial effects to the host, there is a small body of evidence which suggests that some probiotics can confer deleterious effects.

Potentially serious side-effects of probiotic therapies include the development of sepsis (Boyle et al., 2006); initiation of an extreme inflammatory response (Liong, 2008); growth of foreign bacterial colonies which inhibit normal colonization of other microbiota (Neu, 2007); presence of virulence factors within strains of probiotic bacteria (Wassenaar and Klein, 2008); translocation of live bacteria into local tissues (Liong, 2008) and the transfer of resistance genes throughout bacterial populations as a result of anti-microbial factors released by the bacteria (Honeycutt et al., 2007). As intestinal mucositis is commonly associated with reduced immune capacity and gut function, patients may be at an increased risk of side-effects such as the development of bacteraemia, alteration of the gut microbiota, and uncontrolled inflammation. These concerns prompt consideration of alternative, probiotic-based products such as inactivated/dead bacteria (Kataria et al., 2009) or the factors secreted by the live bacteria (Prisciandaro et al., 2009). These alternate forms do not contain live bacteria, and as such there is a reduced risk of bacteraemia and sepsis.

Furthermore, as probiotics are commonly regarded as a dietary supplement (rather than as a pharmaceutical or biological treatment), it is not a requirement that they demonstrate purity or potency prior to commercial availability (Boyle et al., 2006). The requirements which must be met before a probiotic is commercially available differs between regions. In Europe, only dietary supplements which are intended for use by young children undergo screening, while in the United States and Australia, probiotics intended for specific intestinal disorders are recognized as biological treatments and are reviewed by the Food and Drug Administration Regulatory Authority (Boyle et al., 2006). In light of the risks outlined above, more rigorous screening and safety testing of probiotic bacteria must be carried out as a part of any investigations using the bacteria as a therapy.

PROBIOTICS IN INTESTINAL MUCOSITIS

Animal Models of Intestinal Mucositis

Numerous established animal models of intestinal mucositis can be used to screen potential treatments such as probiotics (Tooley et al., 2006a; Gibson et al., 2007; Yeoh et al., 2007). A small number of studies have recently emerged which demonstrate the protective and therapeutic potential of probiotics. von Bultzingslowen and colleagues reported that *L. plantarum* 299v treatment via drinking water increased feed intake and body weight of 5-FU treated rats, and reduced the 5-FU-induced increase in the total number of facultative anaerobes in the intestine (Von Bultzingslowen et al., 2003). In contrast, diarrhoea and bacterial translocation to lymph nodes were not altered by probiotic treatment. Probiotics have also demonstrated efficacy in ameliorating methotrexate (MTX)-induced mucositis. Southcott and colleagues tested cows milk yoghurt fermented with *L. johnsonii* and sheep milk fermented with both *Lactobacillus bulgaricus* and *S. thermophilus* (Southcott et al., 2008).Both treatments protected the duodenum from MTX-induced damage at the histological level, but this protection was not observed in other sections of the small intestine. However, a decreased lactulose/mannitol ratio for probiotic-treated animals indicated improved small intestinal barrier function. These authors did not test the efficacy of yoghurts which had not been fermented with live bacteria, and thus the active component of the treatment

was not determined. *Streptococcus thermophilus* TH-4 has been examined in the MTX and 5-FU models of intestinal mucositis (Tooley et al., 2006a; Whitford et al., 2009). Tooley and colleagues tested live TH-4 at two doses (10^8 and 10^9cfu/ml) delivered daily between 48 hours prior to, and 96 hours post-MTX treatment. A dose-dependent response was observed, with rats given MTX + 10^9 TH-4 displaying similar sucrase levels to non-MTX treated controls, indicating a normalization of intestinal function. Furthermore, the higher dose of TH-4 significantly reduced MPO activity when compared to MTX-treated control animals. These findings contrast with findings of Whitford and colleagues (2009) who examined live TH-4 and its supernatant in 5-FU induced mucositis. In this study, live TH-4 was only able to reduce disease severity scores, while the previously reported increase in sucrase and decrease in myeloperoxidase (MPO, an indicator of tissue damage) activities were not observed. These findings highlight that probiotic effects may vary based on the chemotherapeutic agent and the mechanism of gut damage.

In the 5-FU rat model of mucositis, Mauger and colleagues (2007) reported that administration of either *L. fermentum* BR11, *L. rhamnosus* GG or *B. lactis* Bb12 at 10^6 cfu/ml had no effect on MPO and sucrase activity, or on histological damage scores. The authors suggested the absence of a probiotic effect may have been due to the low dosage administered; a notion supported by the findings of Smith and colleagues (2008) who investigated *L. fermentum* BR11 in synbiotic combination with the prebiotic fructooligosaccharide. Although this synbiotic combination was unable to confer any therapeutic benefit, individual administration of *L. fermentum* BR11 reduced 5-FU-induced inflammation in the jejunum. The probiotic combination VSL#3 was investigated in rats treated with irinotecan (Bowen et al., 2007). Rats received VSL#3 either pre-irinotecan treatment, post-irinotecan treatment, or both. Only the latter treatment was able to confer protection against chemotherapy-induced symptoms. Pre- and post-chemotherapy VSL#3 administration increased epithelial cell proliferation, reduced epithelial cell apoptosis, and prevented water and electrolyte imbalance, subsequently preventing diarrhoea. These effects were associated with a reduction of the irinotecan-induced increase in goblet cell number and mucin secretion (Bowen et al., 2007), providing further evidence of the benefits that can be achieved using a probiotic combination. To this end, a mechanism to determine the most active strains may facilitate the development of more efficacious probiotic therapies for intestinal mucositis.

Clinical Trials

There is currently a shortage of well-conducted, large, randomized, double-blind, placebo-controlled trials which investigate the efficacy of candidate probiotic species in intestinal mucositis. This is most likely a result of conflicting data obtained from animal trials and the absence of detailed in vitro studies. Delia and colleagues (2007) investigated the use of VSL#3 as a preventative treatment for radiation-induced diarrhoea. The study involved 490 patients who underwent adjuvant postoperative radiation therapy following surgery for cervical, rectal or sigmoid cancer. Subjects received one sachet (450 billion live bacteria/g) *b.i.d.* for the duration of radiation therapy. Treatment with VSL#3 reduced the incidence of diarrhoea (particularly severe cases) and the number of bowel movements per day when compared to placebo. This study demonstrated that probiotic bacteria can act as a simple, safe, and effective method of protecting cancer patients from radiation-induced diarrhoea. The results of this trial, combined with the earlier results of Bowen et al. (2007) and other clinical trials using this combination in intestinal disorders (Kim et al., 2005; Miele et al., 2009), suggesting that VSL#3 is an ideal candidate for clinical trials in the setting of chemotherapy-induced intestinal mucositis.

SUMMARY

Despite continued research, there remains no definitive treatment for chemotherapy-induced mucositis. Recent findings which demonstrate an involvement of the intestinal microbiota in the condition and the ability to manipulate this environment with probiotic bacteria (Sartor, 2004) present a viable option for the development of either a probiotic-based therapy or prophylactic-treatment.

Appropriate probiotic administration has the potential to decrease the severity of intestinal mucositis. A number of potential mechanisms have been indentified, including reduction of pro-inflammatory cytokine secretion and gene expression, release of anti-inflammatory cytokines, inhibition of inflammatory pathways, improvement of barrier function, maintenance of mucin secretion, prevention of epithelial cell apoptosis and oxidative damage, and the elimination of pathogenic bacteria. However, these mechanisms have been observed predominantly in other disease settings and few studies have investigated the efficacy of probiotics in mucositis. Currently, identification of the most suitable probiotic strains should be the target for research in this field. While many probiotics have multiple beneficial effects, it remains unlikely that a single strain will be sufficient to counteract such a multi-faceted condition. The microbial composition of the host may also affect probiotic efficacy. This review proposes the promising efficacy of probiotic combinations, and the authors predict that a strategically-selected combination of strains may be most efficacious in this disorder.

The authors suggest a number of directions for future research. Primarily, the capacity for probiotics to exert their beneficial effects in the setting of chemotherapy-induced mucositis must be determined. Although the current review provides evidence for efficacy, there remain only a small number of confirmation studies which have been performed in mucositis models. Future studies should isolate the strain-specific mechanisms of ameliorating damage, and elucidating the effects of chemotherapy on probiotic cell viability. The effects of chemotherapeutic drugs on the microbiota suggest that their administration may inhibit the survival and thus the effectiveness of live probiotic-based therapies. Furthermore, the use of probiotic-based

secreted factors, rather than live bacteria, remains an area of future promise. The absence of live cells reduces the need to maintain cell viability and could be particularly beneficial for chemotherapy patients at increased risk of infection due to impaired intestinal barrier function. Probiotics could potentially be employed as prophylactic treatments which inhibit the development of mucositis or as a post-treatment to facilitate the recovery process.

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